Allele frequency of the short tandem repeat locus human lipoprotein lipase(LPL) gene by polymerase chain reaction in the Korean population

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CONTENTS

Ⅰ. INTRODUCTION

Gene analysis was introduced to forensic individual identification of mass disaster and paternity testing,[14,16,28] since restriction fragment length polymorphism (RFLP) was discovered in the mid 1980's. It was admitted as identificational trust more than other conventional identification methods. Recently, individual identification of a STR-containing loci has been actively used in forensic individual identification, because short tandem repeat(STR) locus of which repeatitive sequence is 2-5bp has high efficiency of amplification by polymerization chain reaction (PCR), high power of discrimination and also is easy to be detected through silver stain.[5,21,25,29]

Human Lipoprotein lipase(LPL) gene area is one of STR locus and located on the human chromosome 8q22. The rank of repeatitive sequence is [AAAT]n and molecular size of LPL is 105-133bp. There are 8 kinds of allele 7, 8, 9, 10, 11, 12, 13, 14.[27]

STR locus has a demerit that it has low contribution about individual identification when it is used alone in gene analysis, because it has small number of allele, low heterozygosity and high probability which has a same genotypes between individuals. By analysis of many STR locus at the same time, it can be lowered the possibility that some genotype is accorded with other's one accidentally. But it is a essential prerequisite to get the gene distribution because the distribution of allele is different according to ethnic group[3,22,23,24].

In this study, the distribution of allele and genotype of LPL STR locus in Korean population was obtained to use the basic data for the individual identification and paternity testing

Ⅱ. MATERIALS AND METHODS

Subjects

1.5~2.0㎖ of blood obtained from 201 unrelated Koreans residing in the Kwang-ju city.

DNA extraction

Genomic DNA was prepared from white blood cell by salt precipitation of protein and treated by 100% ethanol precipitation conventionally[1,2,15].

The purified DNA quality and quantity were estimated with UV visible spectrophotometer. Purity of DNA was determined by evaluatly the A260/A280 ratios

DNA amplification

It was used 1㎛ each primer for Human LPL locus(GZ-14 = ATCTGACCAAGGATAGTGGG ATATA, GZ-15 = CCTGGGTAACTGAGCGAG ACTGTGTC), 2mM each dNTP(Promega®), 0.01 unit of Taq polymerase(Promega), 500mM KCl, 100mM Tris-HCl, Ph 9.0, 1% Triton X-100 and 15mM $MgCl_2$ in 25㎕ final reaction volume to amplify 20~50ng of genomic DNA. One drop of mineral oil was overlayed the reaction mixtures in thin walled tubes.

The amplification parameters were 96℃ for 2min, 1cycle; 94℃ for 60 sec, 60℃ for 60sec, 70℃ for 90sec, 10cycles; 90℃ for 60 sec, 60℃ for 60sec, 70℃ for 90sec, 20cycles, 60℃ for 3min, 1cycle in a GeneAmp PCR System 480 Thermal cycler.(Perkin Elmer Co.)

Electrophoresis of DNA amplification products.

Amplified LPL allelic profiles were analyzed on 4% denaturing polyacrylamide gel containing 7M urea after mixed 2.5㎖ of 2X loading solution(10mM NaOH, 95% formamide, 0.05% bromo-phenol blue, 0.05% Xylene cyanol FF, Promega®)

Electrophoresis was conducted for 1 half hour in 1X TBE buffer, LPL alleles resolved on vertical PAGE gel were visualized after silver staining.

The silver staing procedure was as follows;

Step	Solution	Time
a. fixing	fix/stop solution	20 min
b. washing	deionized H2O	2 min ×3
c. staining	staining solution	30 min
d. washing	deionized H2O	10 sec
e. developing	developer solution (4-10℃)	5 min
f. stoping	fix/stop solution	5 min
g. washing	deionized H2O	2 min
h. dry		

Statistical evaluation

Evaluation of the Hardy-Weinberg equilibrium was based on comparisons of observed and expected genotypes, using chi-square test.

The power of discrimination(PD)[11,18,19] and allelic diversity value(h)[11,18] were calculated using following formula;

$PD = 1-\Sigma Pj^2$(Pj : genotype frequency)

$h = (1-\Sigma Xi^2)(N/N-1)$

(Xi : allele frequency, N : sample size)

Ⅲ. RESULTS

After polyacrylamide gel electrophoresis of PCR products, the isolated bands which had individual allelic gene were observed by silver staining.

Allelic genes in LPL locus were observed of 9, 10, 11, 12, 13. and not of 7, 8, 14.

The most frequent allele was 10. The distributions of observed allele frequencies and number of alleles for the LPL STR locus in the

Korean population are shown in table 1 and figure 1.

Table 1. Distribution of LPL allelic gene in 201 Korean

N	201	
allele	Number of alleles observed	allele frequency
7	0	0
8	0	0
9	8	0.020
10	287	0.714
11	40	0.100
12	66	0.164
13	1	0.002
14	0	0
all	402	1
h	0.454	

N : number h : allelic diversity value

Table 2. Distribution of LPL genotype

Genotypes	Number ovserved	Frequency
9-9	0	0.000
9-10	8	0.040
9-11	0	0.000
9-12	0	0.000
9-13	0	0.000
10-10	95	0.473
10-11	35	0.174
10-12	53	0.264
10-13	1	0.005
11-11	0	0.000
11-12	5	0.025
11-13	0	0.000
12-12	4	0.020
12-13	0	0.000
homozygote	99	0.493
heterozygote	102	0.507
total sample	201	1.000
PD	0.674	

PD ; Power of discrimination value
$=1-\Sigma Pj^2$, Pj ; genotype frequency

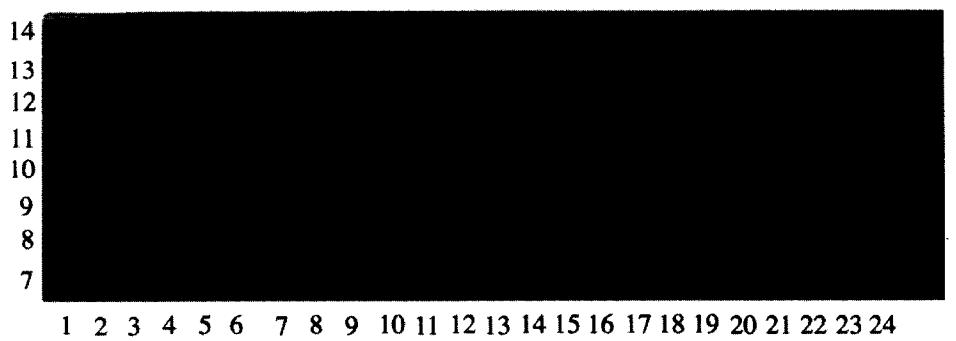

Figure 1. Observed alleles and genotypes in LPL locus
lane 1, 10, 17, 24 : allelic ladder(Promega®), lane 12, 20 : genotype 9-10, lane 4, 5, 8, 11, 15, 18, 21, 22 : genotype 10-10, lane 3, 7, 13, 19 : genotype 10-11, lane 2, 6, 9, 14, 23 : genotype 10-12, lane 16 : genotype 10-13

Furthermore, allelic diversity value was 0.454. The total 7 genotypes were founded in this study. Most common genotype was 10-10 and 10-13 was the only one. Power of discrimination value(PD) was 0.674 and heterozygosity was 50.7%(Table 2, Figure1)

Ⅳ. DISCUSSION

In individual identification and parentage testing, the selection of locus with high heterozygosity is essential. Because forensic samples are mostly corrupted and only a small amount of modified DNA can amplified, the analysis of STR locus by PCR method is very efficient for individual identification. High ploymorphic STR locus is a good marker and contains 2-5 repetitive base pairs and easy to interpret the type of allele[6,8,12,26]. STR locus has individually different in allele frequency of genotype, heteroz ygosity and genetic distance. so it used for individual identification.

In this study, the allele and genotype of LPL STR locus from 201 Korean population was confirmed(table 1,2). A total of 5 Alleles were founded, including alleles 9, 10, 11, 12, 13. But 7, 8, 14 alleles were not detected. A quantitative comparison of allele frequencies for the LPL locus between this study and Japanese population study(n=337) are showed that no significant differences were observed[4]. This fact showed that genetic distance between two ethnic groups was very close. On the other hand, comparing with 3 American ethnic group (African-American, Caucasian-American, Hispanic-American, 219, 204, 210 respectvely), observed alleles in American were similar to Korean, but it was different in 10, 11, 12 allele frequencies.

Authors expected obvious difference of genetic diversity between Oriental and Westerner, but it

Table 3. Allelic frequency of LPL locus in 7 race groups

Koreans			Americans (African)			Americans (Caucasian)			Americacs (Hispanic)			Japanese		Chinese		Hungarian	
homozygotes 99 heterozygotes 102 totalsamples 201			homozygotes 67 heterozygotes 152 total samples 219			homozygotes 47 heterozygotes 157 total samples 204			homozygotes 66 heterozygotes 144 total samples 210			total samples 337		total samples 100		total samples 223	
allele	AF	N	allele	AF	N	allele	AF	N	allele	AF	N	allele	AF	allele	AF	allele	AF
7	0	0	7	0	0	7	0	0	7	0	0	7	0.003	7	0	7	0
8	0	0	8	0.002	1	8	0.002	1	8	0.002	1	8	0	8	0	8	0
9	0.020	8	9	0.146	64	9	0.047	19	9	0.029	12	9	0.001	9	0	9	0.04
10	0.714	287	10	0.370	162	10	0.412	168	10	0.502	211	10	0.712	10	0.855	10	0.408
11	0.100	40	11	0.151	66	11	0.287	117	11	0.224	94	11	0.101	11	0.02	11	0.267
12	0.164	66	12	0.272	119	12	0.203	83	12	0.207	87	12	0.180	12	0.125	12	0.249
13	0.002	1	13	0.059	26	13	0.049	20	13	0.033	14	13	0.003	13	0	13	0.029
14	0	0	14	0	0	14	0	0	14	0.002	1	14	0	14	0	14	0.007
All	1	402	All	1	438	All	1	408	All	1	420	All	1	All	1	All	1

was similar genetic diversity among the American, Hungarian, Chinese and Korean population(table 3)[7,13,27]. Probably it was resulted from small sample size. So we need to add the sample size in the future.

Korean heterozygosity of LPL was different to American one. Observed heterozygosity in Korean population was 0.507 and 0.694, 0.769, 0.686 in 3 American ethnic group[27].

PD of LPL locus was 0.674. This is lower than PD of Hum TH01 locus (0.80)[9,10], HUM VWA locus (0.97)[11], F13A01 locus (0.804)[20], F13B locus (0.610)[17]. It suggested that several STR loci should be detected simultaneously for accurate individual identification.

Allele frequency is the degree of expression of allele from certain locus, and this is the basic index of measure the genetic diversity of genetic group. Individual identification can be performed as analysis of individual genomic variation by using of allelic frequency. But it must be a prerequisitive condition before identification to reveal whether there is a reliance of allelic frequency of certain locus in genetic group, a similar frequency of Hardy-Weinberg equilibrium in the next generation. For obtaining this object, genetic analysis of numerous sample size is needed.

In this study, 5 alleles and 7 genotypes among 14 genotype of LPL locus were observed. PD was 0.674 and observed heterozygotes were 102, so heterozygosity was 50.7%. It was based on Hardy-Weinberg equilibrium. This result was verified by chi-square test to examine the significance about observed value of genotype and expected value of frequency of allele as in the table 4. It was not statically significant.

VNTR locus, so called Minisatellite, is also being used as a good paternity test marker since it contains many of alleles and high heterozygosity. But VNTR locus has a high molecular weight in compare with STR locus, so it is

Table 4. Hardy-Weinberg equilibrium test of LPL genotypes

Genotypes	No. observed(F)	No. expected(F)	χ^2
9-9	0(0)	0(0.08)	0.08
9-10	8(0.04)	0.029(5.741)	0.889
9-11	0(0	0.004(0.804)	0.804
9-12	0(0)	0.007(1.319)	1.319
9-13	0(0)	0(0.016)	0.016
10-10	95(0.473)	0.51(102.469)	0.544
10-11	35(0.174)	0.143(28.703)	1.381
10-12	53(0.264)	0.234(47.073)	0.746
10-13	1(0.005)	0.003(0.574)	0.316
11-11	0(0)	0.01(2.01)	2.01
11-12	5(0.025)	0(6.593)	0.385
11-13	0(0)	0.033(0.08)	0.08
12-12	4(0.02)	0(5.406)	0.366
12-13	0(0)	0(0.132)	0.132
homozygote	99(0.493)	109.965	1.093
heterozygote	102(0.507)	91.035	1.321
total	201(1)	201	9.068776

F = frequency, $0.05 < P < 0.90$, d.f. = 13

difficult to detect the VNTR locus occationally, especially in degraded samples. While on the other hand, is easy to detect the STR locus (so called microsatellite) from the degraded one. Power of discrimination of STR locus is lower than VNTR locus. Therefore, for increasing the power of discrimination, it is recommanded to examine many of the VNTR locus and STR locus at the same time.

V. CONCLUSIONS

From the 201 unrelated Korean population in Kwang-ju, the blood was collected. The DNA was extracted, the LPL gene of STR locus was amplified by PCR, and then silver staining was performed after polyacrylamide gel electropho-

resis for establishing the Korean population data. Obtained results were as follows.

(1) 5 alleles and 7 genotypes of LPL were detected, heterozygosity, allelic diversity value and power of discrimination were 50.7%, 0.454, 0.674 respectively.
(2) The observed alleles were 9, 10. 11, 12, and 13, and allelic frequency of them were 0.020, 0.714, 0.100, 0.164, 0.002 respectively.

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국문초록

한국인에서 중합효소연쇄 반응법에 의한 STR 유전좌위 LPL의 유전자빈도 검색

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한국인 집단에서 개인식별의 기초자료로 활용하고자 한국인 201명을 대상으로 STR 유전좌위 중 하나인 LPL 유전좌위의 유전자 빈도 및 유전자형 분포를 구하였다.

혈액으로부터 추출한 핵 DNA를 중합효소연쇄반응으로 증폭시키고 폴리아크릴아마이드 겔 상에서 전기영동하여 은염색한 후 관찰하여 다음의 결과를 얻었다.

1. 한국인 집단 201명의 LPL 유전자에서 5개의 대립유전자, 7개의 유전자형을 검출하였으며, 이형접합도는 50.7%로 나타났고 대립유전자다양성(allelic diversity value)은 0.454, 개인식별력(PD)은 0.674를 보였다.
2. 대립유전자 및 유전자빈도는 9, 10, 11, 12, 13 대립유전자에서 각각 0.020, 0.714, 0.100, 0.164, 0.002로 나타났으며, 대립유전자 7, 8, 14는 관찰되지 않았다.

이상의 결과를 볼 때 한국인 집단에서 STR LPL 유전좌위의 유전자빈도는 친자감정 등 개인식별에 유용하게 사용할 수 있으나 감정실무에 응용시 다수의 STR 유전좌위 및 VNTR 유전좌위의 분석을 병행하여야 할 것으로 사료된다.